

Effect of high-pressure hot water washing treatment on fruit quality, insects, and disease in apples and pears

Part II. Effect on postharvest decay of d'Anjou pear fruit

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Received 14 April 2005; accepted 15 January 2006

Abstract

A hot water pressure process (HWP) was evaluated for its effect on conidia of *Penicillium expansum* and on development of blue mold, gray mold, and mucor rot of d'Anjou pear fruit. Spores were removed from the water system through dilution and also as a result of hot water in the system that was lethal to the spores. When the system was heated, viable spores were not detected after 2–4 h of operation. Reductions in decay in the HWP system were 36, 29, and 13% for *Botrytis cinerea*, *Mucor piriformis*, and *P. expansum*, respectively. The response of *P. expansum* appeared related to the length of time fruit was in cold storage. Heat injury was observed on fruit treated with 40 and 50 °C water but not on fruit at 30 °C nozzle temperature. The HWP system described in this study should be considered as a component of an integrated decay control strategy.

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Keywords: *Pyrus communis*; *Penicillium expansum*; *Botrytis cinerea*; *Mucor piriformis*; Blue mold; Gray mold; Mucor rot; Integrated control

1. Introduction

Recently, reductions in postharvest decay and insect infestations with hot water brushing have been reported for several crops (Fallik et al., 1999, 2000; Porat et al., 2000; Smith and Lay-Yee, 2000). Winter pears often are stored for 9 months or longer at –1 °C (Spotts and Sanderson, 1994), and lengthy storage is associated with increased decay (Sommer, 1992). Blue mold (*Penicillium expansum*), gray mold (*Botrytis cinerea*), and mucor rot (*Mucor piriformis*) are the main decays of pear (Bertrand and Saulie-Carter, 1980; Lennox and Spotts, 1997; Sanderson and Spotts, 1995; USDA, 1971) and are controlled by an integrated strategy involving pre- and postharvest fungicides, biocontrol, sanitation, careful

fruit handling, harvesting at optimum maturity, and nutrition (Chand-Goyal and Spotts, 1996; Kupferman et al., 1995; Sugar et al., 1994). Hot air treatment (27 °C for 2 and 3 days) reduced side rot caused by *Phialophora malorum* and mucor rot, did not affect gray mold, and increased blue mold (Spotts and Chen, 1987). Heat treatment of fruit prior to inoculation decreased susceptibility of wounds to infection by both *P. expansum* and *P. solitum* (Spotts et al., 1998). Accumulation of tannin, callose, pectic substances, and suberin preceded or coincided with the increase in resistance to decay. Hot water at 47 °C controlled alternaria rot of Spadona pears but enhanced development of *P. expansum*, and at 50 °C caused skin injury (Ben-Arie and Guelfat-Reich, 1969). Pierson (1968), reported that 54.4 °C for 240 s or 57.2 °C for 60 s was required to completely inhibit germination of *P. expansum* spores. Heat sterilization of dump tank water in commercial packinghouses is effective and requires heating to 54.4 °C

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for 20 min (Spotts and Cervantes, 1985). In a preliminary study on the effect of water pressure without heat on decay, blue mold incidence and severity increased in direct proportion to pressure at the nozzles that apply water to the fruit (unpublished data). Thus, any pressurized system must include procedures to control decay.

The objectives of this study were to (i) determine the effect of a hot water pressure system (HWP) on conidia of *P. expansum*, and (ii) to evaluate the HWP process for effect on blue mold, gray mold, and mucor rot of d'Anjou pear fruit.

2. Materials and methods

2.1. Description of hot water pressure (HWP) system

The high-pressure hot water washing system consisting of a boiler, hot water mixing tank, contact loop, heat exchanger, high-pressure pump, spray tank, high-pressure spray manifold, and low-pressure fresh water spray manifold has been previously described (Bai et al., 2006). System temperature was measured by thermocouples every second, averaged over a 7 s period, and logged to a computer data file as described previously (Bai et al., 2006).

2.2. Effect of HWP on conidia of *Penicillium expansum* and blue food coloring to measure dilution

Penicillium expansum isolate 46 was grown in Petri dishes on acidified potato dextrose agar (APDA). Conidia were harvested from 7-day-old cultures with sterile distilled water (SDW) plus 0.01% Tween 80. The spore suspension was vortexed and sonicated to breakup conidial chains. Spore concentration was determined with a hemacytometer. The concentrated conidial suspension was added to the HWP water (temperature at 30 °C, pressure at 551.6 kPa) to achieve an initial concentration of 20,000 spores per milliliter in the system. Blue food coloring (Flag blue 47114, Crescent Foods, Seattle, WA) also was added to the system when spores were added to achieve an initial absorbance of 5.0 ± 0.2 . Water from nozzles spraying onto the fruit was sampled 60, 300, and 900 s after spores were added, then every 900 s for 2 h. Sample (100 μ L) was diluted 1:49 with SDW and 200 μ L were plated on triplicate dishes of APDA. Dishes were incubated at 20 °C, and colonies of *P. expansum* were counted after 48 h. The absorbance of the sample was measured at 630 nm in a spectrophotometer (Spectronic 20, Bausch and Lomb, Rochester, NY).

After the last sample was removed, the temperature of the system was adjusted to achieve a temperature of 40 °C at the nozzles. Spores and blue food coloring were added to the system as described above. Samples were taken, plated on APDA, and absorbance determined as described above. Contact loop (Bai et al., 2006) water temperature was 60 °C in both experiments.

2.3. Effect of HWP on multiple additions of *P. expansum* spores

P. expansum was cultured and spores harvested as described previously. At time 0 (before addition of spores), nozzle water was sampled, concentrated spore suspension was immediately added to the HWP system. Every 0.5 h for 6 h, water was sampled, then spores were added. Samples were diluted, plated, and counted as described above. The experiment was repeated with nozzle water temperature/pressure at 30 °C/552 kPa and 30 °C/496 kPa and twice without any heat in the system (cold water control) where nozzle water temperatures/pressures were 14 °C/552 kPa and 25 °C/524 kPa. Contact loop water temperatures were 61.3 and 62.6 °C in the first and second hot water experiments, respectively. Concentrated spore suspensions varied with each experiment and ranged from 2×10^7 to 9×10^7 conidia per milliliter.

2.4. Effect of HWP on decay of d'Anjou pear fruit

d'Anjou pear fruit were surface sterilized with 0.5% NaOCl, then puncture-inoculated with 2000 spores milliliter of the main decay pathogens, *B. cinerea*, *M. piriformis*, *P. expansum*, and a sterile distilled water control. A metal tool that made a circular wound of 3 mm depth \times 6 mm diameter was dipped in spore suspension and used to make two wounds on each fruit. Spores were obtained from 8-day-old cultures of *M. piriformis* and *P. expansum* and 15-day-old *B. cinerea* growing on APDA. Three 19 kg replicate boxes of puncture-inoculated fruit of each pathogen or non-inoculated control were run through the packingline with and without the HWS in operation. Fruit were placed on the rollers exiting the dump tank and removed immediately after passing under the hot water nozzles. The packingline was rinsed with tap water between each group of fruit inoculated with different fungi. Treated fruit were placed in cardboard fruit boxes with perforated polyethylene liners and stored at -1 °C. Decay was evaluated after 1, 2, and 3 months for *M. piriformis*, *B. cinerea*, and *P. expansum*, respectively. The experiment was replicated four times. Dates and HWP system operating conditions are reported in Table 2.

3. Results

3.1. Effect of HWP on conidia of *Penicillium expansum* and blue food coloring to measure dilution

At both 30 and 40 °C, absorbance decreased by 57% after 300 s and by 61–67% after 900 s (Table 1). After 2 h, absorbance decreased 81–97% compared to the original color. Spore concentration decreased 90–95% after 300 s and 98–100% after 900 s (Table 1). After 1 h, no viable spores were detected at 30 °C, and at 40 °C, spore concentration was reduced 99.6–100%.

Table 1

Effect of hot water pressure system on survival of spores of *Penicillium expansum* and dilution of blue food dye

| Time after spores and dye added (s) | 30 °C | | 40 °C | |
|-------------------------------------|--------|------------|--------|------------|
| | CFU/mL | Absorbance | CFU/mL | Absorbance |
| 60 | 5083 | 4.80 | 89875 | 5.20 |
| 300 | 500 | 2.08 | 4083 | 2.24 |
| 900 | 0 | 1.57 | 1583 | 2.00 |
| 1800 | 75 | 1.10 | 250 | 1.82 |
| 2700 | 75 | 0.78 | 75 | 1.65 |
| 3600 | 0 | 0.56 | 333 | 1.48 |
| 4500 | 0 | 0.40 | 0 | 1.35 |
| 5400 | 0 | 0.30 | 250 | 1.21 |
| 6300 | 0 | 0.21 | 0 | 1.09 |
| 7200 | 0 | 0.15 | 0 | 1.00 |

All values were at spray nozzle. Pressure was 552 kPa at both temperatures. Contact loop and heater temperatures were 60 and 63 °C, respectively. Absorbance measured at 630 nm.

3.2. Effect of HWP on multiple additions of *P. expansum* spores

Spore load in the cold water control varied over the 6-h study but did not show any trends of increase or decrease (Figs. 1A and 2A). Spore load in the system when the HWP was operating fluctuated during the first 3 h in the April exper-

iment (Fig. 1B) and the first hour in the June, 2004 experiment (Fig. 2B) then decreased to low or zero concentrations.

3.3. Effect of HWP on decay of d'Anjou pear fruit

Overall, reductions in decay by the HWP process were 36, 29, and 13% for *B. cinerea*, *M. piriformis*, and *P. expan-*

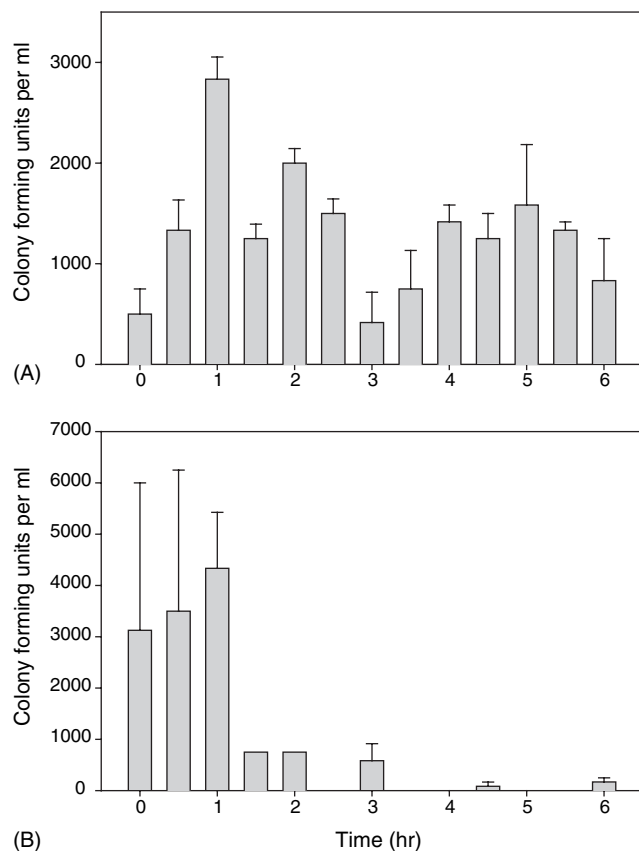


Fig. 1. Colony forming units (CFU) per milliliter of *Penicillium expansum* in the HWP system water without heat (A) and with heat (B). At each time, water sampled first, then spores added. Bars represent means and standard error of means. Experiments done April 2004.

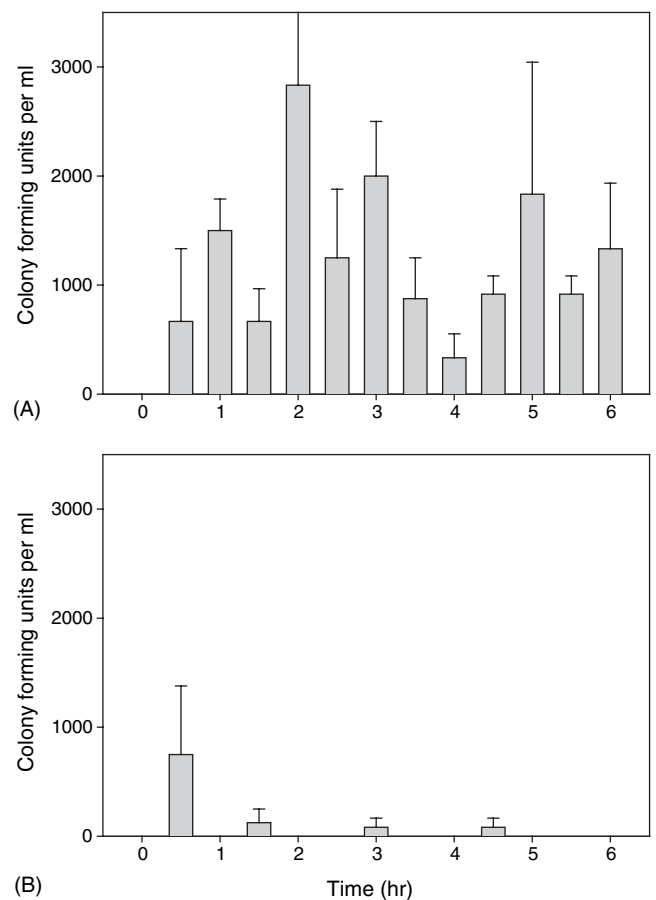


Fig. 2. Colony forming units per milliliter of *Penicillium expansum* in the HWP system water without heat (A) and with heat (B). At each time, water sampled first, then spores added. Bars represent means and standard error of means. Experiments done June 2004.

Table 2
Effect of hot water treatment on incidence of decay of d'Anjou pear fruit

| Date | Wounds infected (%) ^a | | | | | |
|-------------------|----------------------------------|---------|-------------------------|---------|-----------------------------|---------|
| | <i>Botrytis cinerea</i> | | <i>Mucor piriformis</i> | | <i>Penicillium expansum</i> | |
| | Heat ^b | No heat | Heat ^b | No heat | Heat ^b | No heat |
| 13 December 2001 | 9a | 14b | 56a | 81b | 41a | 74b |
| 20 March 2002 | 39a | 81b | 40a | 59b | 84b | 72a |
| 23 September 2003 | 20a | 29b | 18a | 43b | 57a | 75b |
| 16 January 2004 | 67a | 87b | 86a | 98a | 73a | 72a |

^a Numbers followed by the same letter within rows for each pathogen are not significantly different at $P=0.05$ according to a two-sample *t*-test.

^b Water temperature (°C)/pressure (kPa) applied to fruit was 50/552, 40/552, 30/414, and 30/552 for 2001, 2002, 2003, and 2004, respectively. Contact loop temperature in all trials was 60 ± 2 °C.

sum, respectively. HWP-treated fruit had significantly less gray mold in all four experiments than control fruit (Table 2). Similarly, mucor rot was significantly less in HWP-treated fruit than controls in three of the four tests. The effect of the HWP system on *P. expansum* was variable. In two tests, HWP-treated fruit had less decay than control fruit. In the second test, treated fruit had more decay than control fruit, and in the fourth test, there was no significant difference between HWP-treated and control fruit. Heat injury was observed on fruit treated with 40 and 50 °C water but not on fruit at 30 °C nozzle temperature.

4. Discussion

The HWP system was designed to introduce 0.13 L s^{-1} of fresh water. This resulted in dilution of both food coloring and *P. expansum* conidia. However, the decrease in conidia was much greater than the decrease in absorbance of the blue dye, indicating that not only were spores removed from the system through dilution but also that the hot water in the system was lethal to the spores. For example, at 30 °C, the dilution was 57% after 300 s, resulting in a reduction in viable spores from 5083 to 2186. Since only 500 CFU/mL were present, the additional 77% reduction can be attributed to heat. Pierson (1968), reported that 54.4 °C for 240 s or 57.2 °C for 60 s was required to completely inhibit germination of *P. expansum* spores. Since temperature at the nozzles applying water to the fruit surface was only 30–40 °C, it is likely that the spores were killed in the heat tank and contact loop which were about 60 °C with exposure times of 24 and 60 s, respectively. The relationships between inoculum concentration and decay are complex, and threshold spore concentrations have not been established (Spotts, 1986). However, it is important to reduce spore concentrations in packinghouse water systems to the lowest possible level, and the HWP system contributes significantly to this reduction.

When spores were added to the HWP system every 0.5 h, replacement of contaminated water with fresh water caused the spore level to vary somewhat because of uneven mixing throughout the system, but no trend of increase or decrease in spore concentration was observed when the heat was not operating. However, when the system was heated, viable

spores were not detected after 2–4 h of operation. Temperature records showed that the disappearance of viable spores coincided with an increase in temperature of the water in the heat tank and contact loop to levels lethal to spores of *P. expansum*.

The HWP system reduced gray mold, mucor rot, and blue mold by 36, 29, and 13%, respectively. In studies with hot air, *P. expansum* was less sensitive to heat than *B. cinerea* or *M. piriformis* (Spotts and Chen, 1987), and results were similar with hot water (Spotts and Cervantes, 1985). Pear fruit wound-inoculated with *M. piriformis* and dipped in 47 °C water for 0.5 h had 1–5% infected wounds, whereas fruit dipped in water at 21 °C had 90% infection (Michailides and Ogawa, 1989). In a study on the effect of hot water at 47 °C for 420 s on Spadona pear (Ben-Arie and Guelfat-Reich, 1969), decay caused by *P. expansum* increased following heat treatment. In results herein, response of *P. expansum* appeared related to the length of time fruit was in cold storage. When fruit was treated in September and December, 1–3 months after harvest, blue mold was reduced by an average of 25%. Blue mold in fruit treated in January, after 4.5 months of air storage at -1 °C was not significantly different from control fruit. However, when fruit were treated in March after 6.5 months of storage, blue mold increased in the HWP system by 12% compared with the control. Fruit commonly increase in susceptibility to decay as time in storage increases (Sommer, 1992). Control of stem decay of pear, caused mainly by *P. expansum*, depended on a combination of fungicides and heat (Pierson and Couey, 1970).

The HWP process described in this study should be considered as a component of an integrated decay control strategy. The system reduced gray mold and mucor rot as well as blue mold in recently harvested pears. The system cannot be relied on to give adequate decay control alone, and integrated measures are necessary for satisfactory levels of commercial decay control.

Acknowledgements

This project was supported in part by the Winter Pear Control Committee and by USDA ARS Cooperative Agreement Project 5352-43000-009-10S. Use of trade names in this arti-

cle does not imply endorsement by Oregon State University of the projects named or criticism of similar products not mentioned.

References

- Bai, J., Mielke, E.A., Chen, P.M., Spotts, R.A., Serdani, M., Hansen, J.D., Neven, L.G., 2006. Effect of a high-pressure hot water washing treatment on fruit quality, insects, and disease in apples and pears. Part I. System description and the effect on fruit quality of d'Anjou pears. *Postharvest Biol. Technol.* 40, 207–215.
- Ben-Arie, R., Guelfat-Reich, S., 1969. Postharvest heat treatment to control storage rots of Spadana pears. *Plant Dis.* 53, 363–367.
- Bertrand, P.F., Saulie-Carter, J.L., 1980. Mucor rot of pears and apples. *Agric. Exp. Sta. Oregon State Univ. Spec. Rep.* 568, 21.
- Chand-Goyal, T., Spotts, R.A., 1996. Control of postharvest pear diseases using natural saprophytic yeast colonists and their combination with a low dosage of thiabendazole. *Postharvest Biol. Technol.* 7, 51–64.
- Fallik, E., Aharoni, Y., Copel, A., Rodov, V., Tuvia-Alkalai, S., Horev, B., Yekutieli, O., Wiseblum, A., Regev, R., 2000. Reduction of postharvest losses of Galia mellon by a short hot-water rinse. *Plant Pathol.* 49, 333–338.
- Fallik, E., Grinberg, S., Alkalai, S., Yekutieli, O., Wisblum, A., Regev, R., Beres, H., Bar-Lev, E., 1999. A unique rapid hot water treatment to improve storage quality of sweet pepper. *Postharvest Biol. Technol.* 15, 25–32.
- Kupferman, E., Spotts, R., Sugar, D., 1995. Practices to reduce postharvest pear diseases. *Tree Fruit Postharvest J.* 6 (2), 18–23.
- Lennox, C.L., Spotts, R.A., 1997. Botrytis gray mold as a postharvest pathogen in d'Anjou pear. In: Kupferman, E. (Ed.), *Proceedings of the 13th Annual Washington Tree Fruit Postharvest Conference*. Washington State Horticultural Association, Wenatchee, pp. 93–95.
- Michailides, T.J., Ogawa, J.M., 1989. Effects of high temperature on the survival and pathogenicity of propagules of *Mucor piriformis*. *Phytopathology* 79, 547–554.
- Pierson, C.F., 1968. Method for determining the sensitivity of fungus spores to hot water. *Phytopathology* 58, 538–539.
- Pierson, C.F., Couey, H.M., 1970. Control of decay in Anjou pears with hot water and with heated and unheated suspensions of benomyl or thiabendazole. *Phytopathology* 60, 1308.
- Porat, R., Daus, A., Weiss, B., Cohen, L., Fallik, E., Droby, S., 2000. Reduction of postharvest decay in organic citrus fruit by a short hot water brushing treatment. *Postharvest Biol. Technol.* 18, 151–157.
- Sanderson, P.G., Spotts, R.A., 1995. Postharvest decay of winter pear and apple fruit caused by *Penicillium*. *Phytopathology* 85, 103–110.
- Smith, K.J., Lay-Yee, M., 2000. Response of 'Royal Gala' apples to hot water treatment for insect control. *Postharvest Biol. Technol.* 19, 111–122.
- Sommer, N.F., 1992. Principles of disease suppression by handling practices. In: Kader, A.A. (Ed.), *Postharvest Technology of Horticultural Crops*. University of California Publ. 3311, pp. 109–116.
- Spotts, R.A., 1986. Relationships between inoculum concentrations of three decay fungi and pear fruit decay. *Plant Dis.* 70, 386–389.
- Spotts, R.A., Cervantes, L.A., 1985. Effects of heat treatments on populations of four fruit decay fungi in sodium ortho phenyphenate solutions. *Plant Dis.* 69, 574–576.
- Spotts, R.A., Chen, P.M., 1987. Prestorage heat treatment for control of decay of pear fruit. *Phytopathology* 77, 1578–1582.
- Spotts, R.A., Sanderson, P.G., 1994. The postharvest environment and biological control. In: Wilson, C.L., Wisniewski, M.E. (Eds.), *Biological Control of Postharvest Diseases — Theory and Practice*. CRC Press, Boca Raton, pp. 43–56.
- Spotts, R.A., Sanderson, P.G., Lennox, C.L., Sugar, D., Cervantes, L.A., 1998. Wounding, wound healing, and staining of mature pear fruit. *Postharvest Biol. Technol.* 13, 27–36.
- Sugar, D., Roberts, R.G., Hilton, R.J., Righetti, T.L., Sanchez, E.E., 1994. Integration of cultural methods with yeast treatment for control of postharvest fruit decay in pear. *Plant Dis.* 78, 791–795.
- U.S. Department of Agriculture, 1971. Market diseases of apples, pears and quinces. *Agric. Res. Serv. Agric. Handb.* 376, 112.